1. Title of the thesis

"Hydrolytic Reactions of Organophosphate Esters involving C-N-P Linkage"

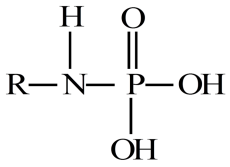
2. Introduction

Phosphate esters are very essential in life processes[1], in living organism for growth, development and maintenance of all plants and animals. These esters are found in nucleoside phosphates (nucleotide) as components of RNA and DNA, in sugar nucleotide for the glycosylation of oligosaccharides, and in proteins involved in intracellular signalling and regulation [2].The hydrolysis of phosphate esters is one of the most fundamental chemical and biochemical reactions[3].The mechanisms of hydrolysis of phosphate diesters are of great interest because phosphate diester linkage is highly stable toward solvolytic cleavage and is widely utilized throughout the nature [4]. Hydrolysis of phosphate ester is ubiquitous in biology, being involved in protein synthesis, energy transduction, and the replication of genetic material [5]. Therefore, phosphate esters have been the subject of extensive experimental[6] and theoretical[7] studies.

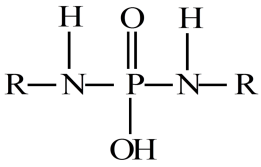
Organophosphorus compounds are of interest to many group of chemists due to their versatile application in various fields, organic chemists investigate the reaction mechanisms to improve synthetic routes to these chemicals[8], biochemists study their relationship to cholinesterase inhibition[9], environmental chemists are interested in these compounds for their pesticidal and insecticidal role, as well as their persistence and overall toxicity in the environment[10]. These are widely used in heavy industries as hydraulic fluid additives, synthetic lubricants, and in the petrochemical industry as plasticizers[11], flame retardants[12]. Despite the extensive production and resultant widespread exposure of environmental compartments to various amounts of these compounds, very few quantitative data on their hydrolytic reactivity are reported in the literature.

Organophosphate esters are the derivatives of orthophosphoric acid and infinite raminifications are possible by making changes in substituent groups attached to phosphorus atom through specific linkages such as C-N-P, C-S-P and C-

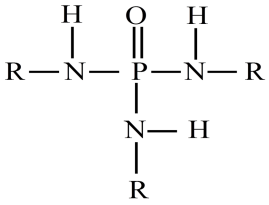
O-P. Phosphoric acid can in principle be esterified at one, two, or three positions, forming phosphate monoester, diester or triester-



Monoester



Diester



Triester

Where R=Aryl or Alkyl group

The study of hydrolysis of organic phosphate esters is useful in various branches of chemistry due to its applications in different fields. Due to this, the phosphate esters have become the subject of research interest to chemists and biochemists. The present work concerns about the synthesis of some phosphate esters and their hydrolytic studies under different experimental conditions such as effect of hydrogen ion concentration, temperature, solvent, ionic strength etc. in detail. Micellar induced reactions, due to their unique functional similarities with enzyme-catalysed reactions, have established an important place not only in chemical science but also in biochemical science. Study of these micellar reactions, especially the kinetic aspects of these reactions, has gained momentum in recent time as these are useful to varied interests. The micelle generally increases the nucleophilic reactivity. There is very few work reported in the literature on the study of hydrolysis of phosphate esters using different types of surfactants. Therefore the work will be carried out further to study the effect of surfactants on the hydrolysis of phosphate esters.

2. A brief review of the work already done in the field

A review of the study of organophosphorus compounds in different fields has been summarized briefly as given below:-

Nome with his research group[13] [2013] have studied intramolecular general base catalysis in the hydrolysis of a phosphate diester and proposed a single –step, concerted SN^2 (P) mechanism involving the attack of solvent water on phosphorus

assisted by intramolecular catalysis by a (weakly basic) pyridine nitrogen acting as a general base. A very limited number of literatures are available related to antimicrobial study of phosphate esters. Adelowo [14] [2012] has synthesized some dialkyl, alkylphosphonates and dialkyl phenylphosphonates and studied their invitro fungicidal activity by using MIC (minimum inhibitory concentration). Jadhav et al[15] [2012] have reported the antibacterial activity of mono and di-2-ethylaniline phosphate by determining the minimum inhibitory concentration for development of novel antibacterial agents with high potency.

Md. Ehtesham Ul Hoque and Hai Whang Lee[16] [2011] have studied kinetics and mechanism of anilinolysis of di isopropyl chlorophosphate in acetonitrile at 55^{o} C. They have proposed a mechanism involving predominant backside nucleophilic attack on the basis of secondary inverse deuterium kinetic isotope effects (DKIEs). Ashkenazi et al [17] [2010] have carried out the mechanism of nucleophilic displacements at phosphorus in chloro-substituted methyl phosphonate esters, P-O Vs P-C bond cleavage by using density functional theory (DFT) study. The results have revealed that sequential addition of chlorine substituent on the methyl phosphonate increases the stability of transition states and intermediates which facilitate P-C bond cleavage. Satnami et al[18] [2010] have studied nucleophilic attack of salicylhydroxamate ion at C=O and P=O centers in cationic micellar media. This study indicates that hydroxamate ions are very strong nucleophiles for nucleophilic attack at the C and P center. The pseudo-first order rate constant surfactant profiles show micelle-assisted biomolecular reaction involving interfacial ion exchange between bulk aqueous media and micellar pseudophase.

Moliner et al[19] [2009] have studied theoretical modelling of the reaction mechanism of phosphate monoester hydrolysis in alkaline phosphatase analyzed by means of hybrid QM/MM simulations. The reaction mechanism obtained is either associative or dissociative, depending on the size of the QM subsystem. The description obtained using the largest QM region agrees with the picture obtained from experimental data. The interpretation of the observed linear free energy relationship in phosphate hydrolysis thorough computational study of phosphate diester hydrolysis in solution has been carried out by Warshel et al [20] [2008]. They

have introduced and improved restrain release approach for evaluating conformational entropies and applied this approach to the system. Tiago et al.[21] [2007], have studied the intermolecular general acid catalysis of the hydrolysis of 2-(2-Imidazolium) phenyl phosphate. They have solved the crystal structure of IMPP and described a bond length-reactivity correlation for reaction of phosphate monoester monoanion. C. L Shina Kamerlin and John Wikie[22] [2007] have investigated the impact of metal ion on the potential energy surface and hence preferred reaction mechanism for simple model for hydrolysis of phosphate ester mono anion.

Alvan C.Hengge and Kerensa Sorensen–Stowell[23] [2006] have investigated thermodynamic origin of the increased rate of hydrolysis of phosphate and phosphorothioate esters in DMSO/water mixtures. The enthalpies of activation for the hydrolysis reaction in two solvents permitted the calculation of the enthalpy of transfer of transition state. This transfer has been found thermodynamically favorable for both the reaction and transition state. Ghosh et al [24] [2005], studied kinetic study of hydrolysis decomposition of organophosphates and thiophosphate by N-hydroxyamides in cationic micellar media. Micellar solutions of sulfobetaine in water ethylene glycol mixtures have been studied by M. L. Moya et al[25] [2005] the kinetic micellar effects were quantitatively explained by using the pseudo phase kinetic model. Chang –Guo Zhan [26] [2004], have performed a series of first principles electronic structure calculations to examine the reaction pathway and corresponding activation free energies for alkaline hydrolysis of representative phosphodiesters.

4. Noteworthy contribution in the field of proposed work

Organophosphates have been utilized in various application since their initial discovery in the early 19th century. The extreme toxicity, broad spectrum activity, and low cost of organophosphorus compounds have made them popular as pesticides[27], insecticides[28] and chemical warfare agent[29]. Methyl parathion, parathaion, and paraoxon etc. are some of the most widely used pesticides in agriculture. Herbicides (tribufos, (DEF) are tricresyl phosphate containing industrial

chemicals[30]. Trisubstituted phosphorus acid (tri aryl or trialkyl phosphate) such as triphenyl phosphate and tri-iso-propyl phosphate used as an antioxidant.

Recently phosphonoformate trianion (PFA) was found to be effective antiviral agents against acquired immune deficiency syndrome (AIDS) under the names of Foscarnet and Foscavir [31]. Foscarnet is also active against herpes simplex virus (HSV) and AIDS related human cytomegalovirus. Two nucleosides analogues, 3-azidodeoxithymidine (AZT) and 2, 3- dideoxycytidine (DDC), have been therapeutically used for treatment of AIDS patients[32]. The development of phosphate ester prodrugs is an interesting approach to increase intestinal absorption of poorly water-soluble drugs. Fosamprenavir is phosphate ester prodrug of amprenavir[33]. Two antiviral phosphate are currently marketed that contain this type of prodrug adefovir dipivoxil and tenofovir disoproxil for the treatment of hepatitis B, antigen-positive chronic hepatitis B[34].

In chemical industry phosphorus compounds are manufactured. Alkylated arylated phosphates such as phosphites, phosphines and related dimeric, ionic forms and utilized widely. Tris (2-chloroisopropyl) phosphate (TCPP), tris (2-chloroethyl) phosphate (TCEP), tris (2-butoxyethyl) phosphate (TBEP), and tri phenyl phosphate (TPP) are mainly used in variety of products, such as plastic materials, varnishes, lubricants, hydraulic fluids[35].Metallocomplexes of dithiophosphate diesters have been employed for many years as lubrication components in many applications including automobile engines[36]. They are also employed as antioxidant, cracking catalysts and impregnating agent. Tris (2-chloroethyl) phosphate (TCEP) is a flame retardant plasticizer used predominantly in rigid polyurethane foam[37].

5. Proposed methodology during the tenure of research work

I. Synthesis of Phosphate esters:-

New organic phosphate esters will be synthesized by the known methods as described in literature[38] which involves the reaction of parent compound with phosphorylating agents such as $POCl_3$, PCl_5 and P_2O_5 in different mole ratios. Formation of solid mass obtained from this reaction will be separated from aqueous

layer. Monoester will go into in aqueous layer and di-and triester will remain in the solid mass. Di- and triester with the solid mass will be treated with alkali solution. After this they will be purified by re-crystallization using alcohol or ammonia solution. Free mono ester obtained in aqueous layer being unstable will be treated with Ba or Na salts.

II. Characterization of Phosphate Esters:

New organic phosphate esters will be confirmed by following steps:-

(i) Melting point of the synthesized esters will be noted.

(ii) Estimation of elements (C, H, N & P) and comparison between observed and expected % of the elements will be done.

(iii) IR spectra will be recorded. The observed frequencies of these functional groups of P=O, C-N, C-H, P-N, O-H, N-H etc. will be compared with reported one.

III. Kinetic study of the hydrolysis of phosphate esters and spectrophotomeric determination of inorganic phosphate:-

Kinetic study of the hydrolysis of these phosphate esters will be done under different experimental conditions as given below:-

(i) Hydrogen ion concentration: - The effect of hydrogen ion concentration will be studied in different acids using HCl, H_2SO_4 by varying their concentration.

(ii) Ionic strength: - A series of kinetic runs will be made at different ionic strength by using mixture of NaCl, KCl etc. with HCl.

(iii) Temperature effect: - To study the activation parameters a series of kinetic runs will be made at different temperatures. Arrhenius parameters obtained from this will be helpful in identifying the nature of the reaction path.

(iv) Solvent effect: - Different solvents with their different composition will be made for studying solvent effects which will be helpful to determine the formation of transition state.

(iv) Substrate concentration: - Three to four different concentrations of substrate will be taken for studying this effect.

(v) Effect of surfactants: - To study the effect of surfactants on the hydrolysis of phosphate esters different surfactant will be used.

The progress of hydrolytic study of phosphate esters will be done by Allen's modified method [39] using spectrophotometer. The inorganic phosphate produced during hydrolysis forms a phosphomolybdate complex which reduces to molybdenum blue by the action of mixture of amidol and sodium metabisulphite solution. The intensity of the blue colour is proportional to the amount of free phosphoric acid.

In the study of micellar induced reactions CMC determination of surfactant solution is an important physicochemical exercise. CMC determination will be carried out by the most frequently used methods as surface tension[40], conductometry [41] etc.

6. Expected outcome of the proposed work

The important organic phosphate esters having C-N-P linkage will be produced with important properties, useful in various fields. Kinetic study of the hydrolysis of these phosphate esters will provide information about the mechanism and rates of reactions which have fundamental importance and provide substantial basis for understanding the reaction of complicated phosphate esters. The study of the mechanism is of equal interest for both physico-organic chemists as well as bio-chemists. The study may provide substantial basis for understanding the reactions of complicated phosphate esters and the ways in which these esters can be used in medicinal, industrial and agriculture branches of chemistry. The surfactants play very important role in determination of chemical reactivity. Therefore the use of surfactants may affect the rate of reaction. The micelle catalysed kinetic study of phosphate esters will contribute significantly in research as well as in industrial field.

7. Bibliography

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